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# Morphometrics of Heterotrigona Itama Nest Components in the Pocut Meurah Intan Grand Forest Park, Aceh Besar Regency, Indonesia

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**Abstract:** Stingless bees (Hymenoptera: Apidae) are social insects that live together in a colony. Stingless bee species are distributed in Indonesia with varying morphometrics of nest components for each species. Detailed descriptions of the morphometrics of H. itama nest components have not been widely reported. Based on the results of previous research, it is stated that kelulut nest architecture varies both between species and within the same species, but what influences this variation and where the variation occurs in detail has not been widely reported. This study aims to describe the morphometrics of the nest components of the stingless bee species of the Heterotrigona itama species in the Pocut Meurah Intan Grand Forest Park, Aceh Besar Regency, Indonesia. This research method uses direct observation of five H.itama nest colonies which includes measurements of each nest component, namely baby cells, pollen pots, honey pots and nest entrances. The results of this study reported that the nest components of five H.itama colonies had varying sizes. The entrance to the H. itama nest is tube-shaped with a tube length of 2.50 - 18.00 cm and a diameter of 1.50 - 4.50 cm. The diameters of pollen pots, honey pots and daughter cells are in the range of 0.95 - 1.14 cm, 0.65 - 1.14 cm and 0.30 - 0.34 cm respectively. The height of the pollen pot, honey pot and daughter cells ranges from 1.24 - 1.64 cm, 0.63 - 1.20 cm and 0.44 - 0.50 cm respectively. From the results of this research, it can be concluded that H.itama found in the Pocut Meurah Intan Grand Forest Park, Aceh Besar Regency, Indonesia has varying morphometrics in each colony.

**Keywords:** Hymenoptera; meliponini; stingless bees

## Introduction

Stingless bees are social insects that are distributed in subtropical and tropical environments. Currently, stingless bees consist of 605 species grouped into 45 genera spread throughout the world (Engel et al., 2023). In Indonesia, there are 46 known species of stingless bees that can be found on the islands of Sumatra and Kalimantan (Kahono et al., 2018).

Heterotrigona itama is a species of stingless bee which is grouped into the order Hymenoptera, family Apidae and subfamily Meliponinae. Heterotrigona itama has a blackish brown body, the body of H. itama is small, slender, with a body length of around 2.5 – 3.25 mm (Erniwati, 2013), has two pairs of wings, with the front wings of H. itama being longer compared to the hind wings (Saifullizan et al., 2021). H. itama is distributed in Indonesia, distributed in the Bangka Belitung Islands (Azizi et al., 2020), South Kalimantan, Hulu Terengganu (Azmi et al., 2019) (Azmi et al., 2019) and Yogyakarta (Trianto & Purwanto, 2020).

Stingless bees including H. itama are social insects that live in colonies. Members of a stingless bee colony consist of three castes, namely the queen, males and worker bees, all living in one nest (Salatnaya et al., 2020). Each caste in a colony of stingless bees has its own task. The queen and male castes play a role in reproduction, while the worker caste has tasks such as defending the nest from enemy attacks. The H. itama worker bee's defense mechanism is carried out by depositing lots of

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sticky resin droplets around the inside and outside of the nest entrance hole. In addition, H. itama worker bees can clean the nest by carrying waste pellets (small leaves/foreign objects) outside the nest. As a result, a pile of rubbish was found in front of the nest entrance (Azmi et al., 2019).

The H. itama nest consists of internal components, namely a honey pot, pollen pot, daughter cells, and an external component, namely the nest entrance. Each nest component has varying sizes (Azmi et al., 2019). The varying sizes of pollen and honey pots in stingless bee hives are influenced by the nesting space. Worker bees tend to occupy most of the space available for storing honey pots, pollen pots and brood cells (Viana et al., 2015).

Several studies have been carried out on the morphometrics of H. itama nests, including research by Azmi et al. (2019) reported that Heterotrigona itama from Kenyir Tropical Park, Tasik Kenyir, Hulu Terengganu had a honey pot height of 20,364 mm and a diameter of 14,023 mm. The average height is 4,663 mm and the diameter is 3,303 mm. The average entrance length is 114,817 mm and the average entrance diameter is 15,807 mm. Meanwhile, the entrance to H. itama nests in South Kalimantan, Indonesia had an average diameter of 1.38 cm and an average door length of 1.85 cm.

Based on the results of previous research, it was stated that the morphometrics of H. itama stingless bee nest components from various locations showed varying sizes of pollen pots, honey pots, brood cells and nest entrances even within the same species. However, what influences this variation and where the variation occurs in detail has not been reported. Until now there have not been many reports about the morphometrics of the nest components (pollen pot, honey pot, brood cells, entrance) of Heterotrigona itama stingless bees in the Pocut Meurah Intan Grand Forest Park, Aceh Besar Regency, Indonesia. It is hoped that the results of this research will provide data related to the development of H. itama stingless bee cultivation programs and can help determine the type of stingless bee itself.

## Method

*Measurement of the nest entrance*

Nest entrances in five Heterotrigona itama colonies that have been cultivated at Tahura PMI. The nests of H. itama colonies are in wooden boxes and tree trunk cavities that have been cut. Measurement of the length and diameter (cm) of the entrance (Febrianti et al., 2020) was carried out using a ruler (Viana et al., 2015).

*Morphometric measurements of nest components*

Morphometric measurements of nest components include daughter cells, pollen pots and honey pots. The number of daughter cells was counted and the length and diameter (cm) of 50 daughter cell pots were measured. The number of honey pots and pollen pots in the nest was also counted and the height and diameter (cm) of each 20 pots were measured (Octoriadi, 2015). Pots containing pollen look lighter while pots containing honey are darker (Taufik, 2021). The pot measurements were carried out using a ruler and calipers. Each nest observation from five H. itama colonies was documented with a camera, and continued with calculations using the Image J application.

*Estimation of the number of daughter cells*

Estimation of the number of daughter cells in five H. itama nest colonies was carried out by calculating the number of daughter cells per layer, the number of layers of daughter cells in one nest chamber and the number of daughter cells per 2x2 $cm^2$ area. The number of daughter cells per area is obtained by calculating the number of cells per layer and then multiplying by the area per layer. To find out the total number of daughter cells in one nest room, the daughter cells are counted per area of each layer in one nest.

*Measurement of physical environmental factors*

The physical environmental factors measured in this research are temperature (Supratman, 2018), light intensity and air humidity (Pujirahayu et al., 2020). Temperature parameters are measured using a thermometer, light intensity is measured using a lux meter and air humidity is measured using a hygrometer.

*Data analysis*

The measurement results from 50 pots of daughter cells, 20 pots of pollen and 20 pots of honey are presented in the form of mean values ($\bar{x}$) and standard deviation (± SD). Next, the data was analyzed using the Statistical Product and Service Solutions (SPSS) version 25 application by carrying out the analysis of variance (ANOVA) test and followed by Duncan's multiple range test at a confidence level of 5%.

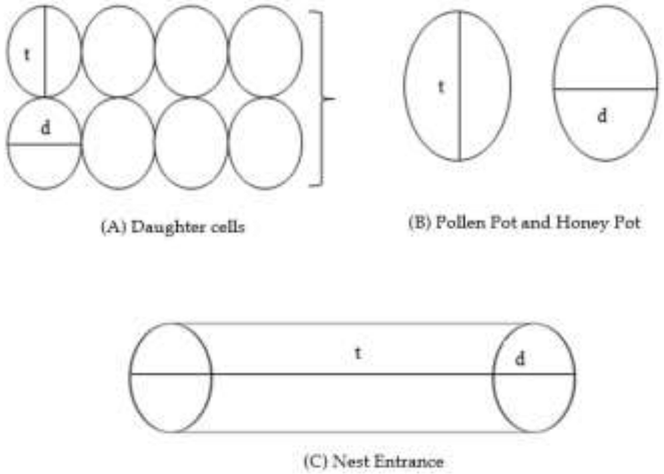


**Figure 1.** How to measure each component of the nest, (A) baby cells, (B) pollen pot and honey pot, (C) nest entrance (Note: t = height and d = diameter)

## Result and Discussion

From the results of observations on H. itama nests in Pocut Meurah Intan Forest Park, Aceh Besar Regency, it was found that each colony has one entrance and the entrance is in the form of a tube with the length of the tube varying from 2.50 cm to 18.00 cm and a diameter ranging from 1.50 cm to 4.50 cm. The largest diameter was at the entrance to the fourth colony, while the smallest entrance diameter was at the third colony (Table 1). The entrance to the kelulut nest, which varies in diameter, is thought to be influenced by the number of worker bee colonies that are very active in foraging for food. According to Li et al. (2021), an entrance with a larger diameter makes it easier for keluluts to enter and exit the nest so that keluluts are more active in foraging activities, on the other hand, the diameter of the door becomes narrower because the keluluts' foraging activity decreases. Meanwhile, according to Viana et al. (2015) a larger entrance diameter is useful for helping worker bees collect pollen, while a small entrance serves as a colony defense from predator attacks. According to Couvillon et al. (2008) the size of the nest entrance can be influenced by predator attacks and the activity of worker bees looking for food.

The nest entrance with the longest size of around 18.00 cm was found in colony four (Table 1). This is thought to be because this colony has a food pot room that is located at a higher height than other colonies. According to Viana et al. (2015) the elevated entrance structure functions for landing of worker bees. High placement of the nest entrance can be associated with a colony defense strategy from predator attacks (Seeley et al., 1982). Meanwhile, according to Sakagami (1978), variations in the size of the nest entrance in kelulut are related to the number of worker bee colonies. Likewise Shackleton et al. (2019) stated that large entrances require a greater number of guard bee members to defend the nest from being damaged by predators. Meanwhile, Cunha et al. (2020)reported that beehives with high population sizes with varying intensity of activity throughout the day indicate variations in the number of bees at the entrance.

This is because the entrance functions as an entry and exit for bees to search for food and general activities such as ventilation, guarding the entrance, and orientation flights. The size of the entrance can also be influenced by the age of the bee colony. Stingless bee colonies that are more than 16 years old have greater height and width of the entrance, namely 1.84 and 2.02 cm respectively compared to colonies less than 15 years old, namely 1.26 and 1. 99 cm. The older the age of the colony can cause the entrance to become larger.

**Table 1.** Differences in nest entrance sizes from five H. Itama colonies

| Diameter (cm)<br>Length(cm) |
|---|
| 2.00 |
| 4.20 |
| 2.00 |
| 8.10 |
| 1.50 |
| 2.50 |
| 4.50 |
| 18.00 |
| 2.50 |
| 7.00 |

The pollen pots found in the five nests of the H.itama colony showed varying numbers, average size, diameter and height of the pots. The first colony had the largest number of pollen pots, namely 81 pots, while the third colony had the fewest pollen pots, namely 36 pots. Each colony has a pot size with a diameter ranging from 0.95 - 1.14 cm and an average pot height ranging from 1.24 - 1.64 cm. Based on statistical results using the Anova test, it shows that there are significant differences in the diameter and height of the pollen pots in the five colonies (sig $< 0.05$). Thus, to see the differences in diameter and height of pollen pots between the five colonies, you can continue with a further test, namely the Duncan test. The results of Duncan's further analysis with 5% confidence showed that the average diameter of the pollen pot in the third, fourth and fifth colonies was significantly different from the first and second colonies. Meanwhile, the average height in the fourth and fifth colonies was significantly different from the first, second and third colonies. This difference may occur due to pollen production, differences in nest shape and size of nesting cavity.

According to Choudhary et al. (2021) and Duarte et al. (2016) variations in nest shape and nest cavity available to bees for making pots can influence the diameter and height of pollen pots. Meanwhile, according to Sihombing (2005), low pollen production causes the pot size to decrease. Low pollen production is caused by a decrease in the productivity of the queen bee and a lack of foraging bees in each hive. The results of calculations and measurements of pollen pots from five H. itama colonies can be seen in table 2.

**Table 2.** Morphometrics of pollen pots in five H. Itama colony nests

| Number of Pots (cm) | Diameter (cm) ($\bar{x} \pm$ SD) | High ($\bar{x} \pm$ SD) |
|---|---|---|
| 81 | 1.14 ± 0,09$^{b}$ | 1.58 ± 0,08$^{b}$ |
| 70 | 1.22 ± 0,10$^{b}$ | 1.64 ± 0,16$^{b}$ |
| 36 | 0.98 ± 0,13$^{a}$ | 1.55 ± 0,43$^{b}$ |
| 54 | 0.95 ± 0,15$^{a}$ | 1.24 ± 0,14$^{a}$ |
| 44 | 1.02 ± 0,13$^{a}$ | 1.28 ± 0,11$^{a}$ |

Note: Different superscript letters (a and b) indicate there is a significant difference (P<0.05).

Apart from pollen pots, this research also calculated the number of honey pots and measured the diameter and height of the honey pots. The results of calculating the most honey pots found in the first colony were 63 honey pots, while the lowest number found in the fifth colony was 20 pots. This occurs allegedly due to the influence of the deviation capacity of the honey pot and feed source. According to Viana et al. (2015) and Pribadi (2021) variations in the number of honey pots in each beehive can be influenced by the food sources available for the bees making pots and the size of the hive's storage capacity for storing pots.

The average diameter and height of honey pots from five colonies showed varying values. The largest diameter of H. itama honey pots was found in the first colony, namely 1.14 ± 0.10 cm, while the smallest average diameter of honey pots was found in the third colony, namely 0.65 ± 0.11 cm. From the results of the Anova test analysis, it shows that the diameter and height of the honey pots are significantly different in the five colonies (sig < 0.05). Given these differences, Duncan's further test was carried out with 5% confidence. Duncan's further test results showed that the average diameter of the honey pot in the third colony was significantly different from the other colonies. Likewise, the average height of honey pots in the third colony was significantly different from the first and second colonies. This difference in size is thought to be because the cavities used as nesting sites for bees in each colony have different sizes. According to Choudhary et al. (2021)differences in the size of the nest cavity affect the diameter and height of the honey pot. The results of calculations and measurements of honey pots from five H. itama colonies can be seen in table 3.

**Table 3.** Morphometrics of honey pots in five H. Itama colony nests

| Number of Pots | Diameter (cm) ($\bar{x} \pm$ SD) | High (cm) ($\bar{x} \pm$ SD) |
|---|---|---|
| 63 | 1.14 ± 0,10$^{b}$ | 1.63 ± 0,10$^{b}$ |
| 47 | 1.02 ± 0,13$^{a}$ | 1.53 ± 0,14$^{a}$ |
| 23 | 0.65 ± 0,11$^{c}$ | 1.21 ± 0,11$^{c}$ |
| 27 | 1.01 ± 0,26$^{a}$ | 1.20 ± 0,09$^{c}$ |
| 20 | 1.05 ± 0,11$^{ab}$ | 1.27 ± 0,09$^{c}$ |

Note: Different superscript letters (a, b and c) indicate there is a significant difference (P<0.05).

From the results of calculating the number of honey pots and pollen pots from the five colonies, it was found that they were 180 pots and 285 pots respectively. This shows that the number of honey pots from the five colonies is less than the number of pollen pots. These results are in accordance with research by Alves et al. (2019) reported that stingless bees have more pollen pots than honey pots. This is because bees need more pollen for their body growth. According to Gowda (2011) bees need a lot of pollen to be used in the development of bees at the metamorphosis stage, namely larvae, pupae, and young bees that are in their body growth period. Taufik (2021) added that pollen and honey are food sources obtained by worker bees from plants. Pollen is obtained by worker bees from the male reproductive organs in flowers and is a source of protein, while honey is obtained by worker bees from the nectar glands in flowers which is a sweet liquid and is a source of carbohydrates. According Sarwono (2001), pollen is a source of protein for bees which can influence the breeding rate and life span of bees. Pollen with a protein content of less than 20% cannot meet the colony's needs for optimal reproduction. A strong colony requires ± 55 kg of pollen per year. If the supply is less than that, bees will use their body protein to continue their functions so that body protein levels can decrease from 54% to 27%.The daughter cell pots found in five H. itama nest colonies showed varying numbers. The highest number of daughter cell pots were found in the first colony, namely 11,468 pots, while the lowest number of pots were found in the fifth colony, namely 6,932 pots. The difference in the number of pots is likely due to the influence of pollen availability and queen bee productivity. According to Agussalim et al. (2017); Neupane & Thapa (2005) and Roubik & Wheeler (1982), the source of pollen availability for bees to forage can influence the number of brood cells. Meanwhile, Sihombing (2005) reported that pollen as a source of nitrogen collected by worker bees will be stored in the nest which is used as a food reserve for the colony and plays a role in the egg production process. Likewise, according to Ghazi et al. (2018), abundant food sources can be useful for providing daughter cells in the nest. According to Agussalim et al. (2017) lack of food obtained from plants can affect the productivity of stingless bee queens to be low. Thus, this will have the effect of reducing the number of baby cell pots in the nest.

The results of measuring the diameter of daughter cells from five H. itama colonies showed varying values, namely in the range of 0.30 to 0.34 cm. Likewise, the height of daughter cells ranges from 0.44 to 0.50 cm. Statistical analysis of the Anova test on the diameter and height of daughter cells showed that there were significant differences between the five colonies. To determine differences in diameter and height of daughter cells in each colony, Duncan's test analysis can be continued. Duncan's test results with 5% confidence showed that the average diameter and height of daughter cells in the fifth colony was significantly different from the other colonies. The difference in the average diameter and height of daughter cells is possibly due to differences in cavity size. According to Barbosa et al. (2013), different nest cavities can produce variations in the diameter and height of the daughter cells in the beehive.

**Table 4.** Morphometrics of daughter cells in five H. Itama colony nests

| Number of Pots | Diameter (cm) ($\bar{x} \pm$ SD) | High (cm) ($\bar{x} \pm$ SD) |
|---|---|---|
| 11468 | $0.33 \pm 0.05^{bc}$ | $0.45 \pm 0.07^{a}$ |
| 7720 | $0.32 \pm 0.03^{abc}$ | $0.44 \pm 0.05^{a}$ |
| 9576 | $0.34 \pm 0.05^{b}$ | $0.50 \pm 0.05^{b}$ |
| 8384 | $0.33 \pm 0.04^{b}$ | $0.46 \pm 0.04^{a}$ |
| 6932 | $0.30 \pm 0.02^{a}$ | $0.44 \pm 0.04^{a}$ |

Note: Different superscript letters (a and b) indicate there is a significant difference (P<0.05).

The daughter cells in the five H. itama colonies are arranged horizontally and in layers to form tiers. The daughter cell layers that form these levels total >10 layers. This shows that the five nest colonies are mature. A mature nest can also be determined by finding one or two potential queen cells that are located differently in the daughter cell layers in each colony. The location of the prospective queen cells is counted from the top layer (Table 5). According to Zohairy (2015), in the lining of the pot of daughter cells, potential queen cells were found, indicating that the beehive was mature. Meanwhile, research by Nicolas (2023) reported that stingless bees have a number of daughter cells consisting of 18 to 34 layers. Likewise, according to Jaapar et al. (2016), a mature stingless bee colony consists of 9 to 14 layers of daughter cells. The number of layers that make up the daughter cells will influence the cell structure. The fewer layers and number of daughter cells, the simpler the arrangement.

**Table 5.** Number of layers of daughter cells and location of potential queen cells

| Number of layers | Place the potential queen cells in the 2nd layer |
|---|---|
| 25 | 10 and 11 |
| 11 | 5 |
| 28 | 3 and 7 |
| 25 | 11 |
| 20 | 8 |

Data from temperature measurements in five H.itama bee nest colonies in the Pocut Meurah Intan Grand Forest Park, Aceh Besar Regency show varying values, namely in the range of 22 -26°C. According to Arkan et al., (2020) temperature can influence the activity of bees in and out of the nest. The higher the temperature, the lower the bee activity will be, so the bees are often in the nest. Basari et al. (2018) explained that a temperature of 30°C can reduce bee foraging activity. Air temperatures below 15°C will cause bees to be lazy about leaving the nest and choose to stay in the nest, so this will cause a shortage of food because worker bees do not look for nectar and pollen. Added that low activity in and out of the nest will have an impact on the low supply of pollen carried by worker bees. This will have an effect on reducing the number of honey pots, pollen pots and daughter cells.

According to Sihombing (2005) environmental factors such as temperature, air humidity and light intensity can influence the number of pollen pots and honey production. These environmental factors will influence life activities and the availability of food sources in nature. Manuhuwa et al. (2013) reported that stingless bees were active at temperatures from 18°C to 35°C. Bee activity is disturbed and decreases if environmental conditions are lower or higher than this temperature. Temperatures that are too high make the bees busy guarding the colony, especially the young, so that they don't die of heat. Meanwhile, if the temperature is low, the activity of worker bees decreases so that their search for pollen and nectar can stop. Bees gather and huddle together to increase the temperature in the hive. Temperatures approaching 0°C can make bees stop their activities (paralyzed), but if the temperature returns to normal, the bees' body activities will gradually return to normal.

Apart from temperature, other factors that can influence the number and size of nest components are light intensity and air humidity. According to Guntoro (2013), high air humidity in the hive due to rainfall will have an impact on reducing the activity of worker bees to collect pollen. Sudarmono & Sahromi (2012) added that low light intensity also has an effect on the activity of worker bees collecting pollen. In the morning, worker bees usually look for pollen and nectar in flowers that have already bloomed. Worker bees will be guided by light to collect pollen. The high or low level of pollen collected by worker bees will have an effect on the number and size of pollen pots, honey and baby cells in the nest.

**Table 6.** Results of environmental factor measurements

| Temperature (°C) | Light intensity (lux) ($x10^2$) | Humidity (%) |
|---|---|---|
| 23 | 42.00 | 80 |
| 25 | 93.60 | 83 |
| 24 | 63.30 | 85 |
| 26 | 93.60 | 85 |
| 22 | 30.20 | 70 |

**Figure 2.** Nest of H. itama

From the results of this research, it was found that even within the same species, the sizes of the pollen pots, honey pots and daughter cells in the five H. itama nest colonies showed varying sizes. This is similar to several studies on the architecture of stingless bees from other species, namely research by Taufik (2021) who reported that the height and diameter of the pollen pot in the stingless bee Geniotrigona thoracica had an average of 27.14 ± 0.74 mm respectively. and 21.80 ± 1.99 mm, while the size of the bee's honey pot with an average pot height of 27.83 ± 1.43 mm and an average honey pot diameter of 18.82 ± 1.11 mm. The variation in pot size is due to the availability of feed. Food sources available in the environment can be influenced by light intensity, air humidity and temperature.

Meanwhile, research conducted by Nicolas (2023) on daughter cells with height and pot diameter ranging from 3.15 - 3.56 mm and 2.07 - 2.37 mm respectively. The difference in size is due to variations in the nest cavity which is used as a nesting place for the stingless bee Tetragonula biroi. Therefore, the results of this research can help identify stingless bee species. The unique characteristics of each hive can also help preserve some bee colonies by arranging additional hives by providing the right type of artificial nesting space to increase food security and can also be used to prepare conservation management plans for stingless bees, especially the H. itama species in the natural environment.

## Conclusion

The morphometrics of the nest components found in the five H. itama colonies varied in size of daughter cells, pollen pots, honey pots and nest entrances. The entrance to the H. itama nest is tube-shaped with a tube length of 2.50 - 18.00 cm and a diameter of 1.50 - 4.50 cm. The diameters of the pollen pot, honey pot and daughter cells were in the range of 0.95 - 1.14 cm, 0.65 - 1.14 cm and 0.30 - 0.34 cm respectively. The height of the pollen pot, honey pot and daughter cells ranges from 1.24 - 1.64 cm, 0.63 - 1.20 cm and 0.44 - 0.50 cm respectively. Variations in the size of H. itama nest components are adjusted to the dimensions of the nest room.

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**Author Contributions**
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**Conflicts of Interest**
The authors declare no conflict of interest.

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